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# Increase of in-dyke alluvial soil fertility, growth and yield of maize (*Zea mays* L.) by potent *Rhodopseudomonas palustris* strains

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## Abstract

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The present study was aimed to evaluate the effects of nitrogen (N) fixing *Rhodopseudomonas palustris* strains, VNW64, VNS89, TLS06 and VNS02 on soil fertility, growth and yield of hybrid maize cultivated on in-dyke alluvial soil (IDAS). The pot experiment followed a completely randomized block design with 10 treatments, 4 replicates for each treatment. The treatments comprised of (i) fertilization with 100% N following recommended fertilizer formula (RFF), (ii) fertilization with 85% N, (iii) fertilization with 70% N, (iv) fertilization with 55% N, (v) treatment i plus a mixture of four purple nonsulfur bacteria (MFPNSB), (vi) treatment ii plus MFPNSB, (vii) treatment iii plus MFPNSB, (viii) treatment iv plus MFPNSB, (ix) no chemical fertilization, and (x) treatment ix plus MFPNSB. The result indicated that treatments supplied with MFPNSB enhanced soil properties, including soil  $\text{pH}_{\text{Water}}$ ,  $\text{NH}_4^+$  content and total N uptake, in comparison with no supplemented bacteria, 6.11–6.15, 16.4–17.7 mg  $\text{NH}_4^+$  kg<sup>-1</sup> and 1.43–5.02 mg N pot<sup>-1</sup> in comparison with 5.74–5.99, 11.3–12.7 mg  $\text{NH}_4^+$  kg<sup>-1</sup> and 0.82–3.11 mg N pot<sup>-1</sup>, respectively, for hybrid maize cultivated on IDAS. Inoculation of MFPNSB on maize seeds contributed to reducing 45% N of RFF, but still caused no decline in the grain yield of maize. At 100% N of RFF, the treatment supplemented MFPNSB possessed a grain yield of 20.2% higher than the treatment without supplemented MFPNSB. Additionally, the treatment fertilized with 85% N of RFF plus MFPNSB, the maize grain yield was increased by 12.5% compared to the treatment fertilized with 100% N of RFF.

## 1. Introduction

Floods accompanied with slurry provides fertility to soil and a clean water source for washing away soil toxins and chemicals remaining on fields. However, floods occurring unpredictably and lacking of control are threats to agricultural systems (Kaur et al., 2020). Therefore, dykes are constructed in order to manipulate water currents, preventing floods from damaging agricultural production (Hsieh and Heibaum et al., 2017). Nevertheless, dykes reduced the nutrient contents for plants, due to the presence of nitrogen (N), phosphorus (P) and potassium (K) amounts in sediments of floods (Walalite et al., 2016). Besides, although dykes control floods, so that farmers can improve their income through intensive farming (Vo, 2021), soil fertility decreases.

Hybrid maize (*Zea mays* L.) is considered to be one of the major cereals utilized as food, livestock feed and industrial materials (Kaul et al., 2019), since maize grains consist of 67–72% starch, 8–12% protein, 2–4% fat, 2–6% carbohydrate, 1–5% oil and 2–3% fiber (Anonyms, 2010). Moreover, the N, P and

K volumes demanded by maize to produce a ton of grain are 216, 31, and 209 kg ha<sup>-1</sup>, respectively (Shehu et al., 2019). For instance, in the case of no fertilization, plants receive nutrition only from soil and have low yield as well (Saddou et al., 2018), while 30% of inorganic N is added, plant yield is raised by 4%, but the loss of N by erosion and greenhouse gases emission is 53% (Chai et al., 2019).

On the contrary, the biological source of N from bacteria is a sustainable solution for plant growth in harsh environments (Khuong et al., 2018; Abadi et al., 2021). Moreover, endophytic bacteria were applied to produce nutrients for maize (Khuong et al., 2022a, 2022b), but purple nonsulfur bacteria (PNSB) possessed the diversity of life mode (Nguyen et al., 2018; Khuong et al., 2020a, 2020b). Among the bacteria used, strains of *Rhodopseudomonas palustris* are potent in reducing both biological and nonbiological stress, supplying N and P, producing plant growth promoting compounds (Khuong et al., 2017, 2020a, 2020b; Sakarika et al., 2020), and reducing greenhouse gases emission (Sakpirom et al., 2017). Simultaneously, strains of *R. palustris* have diverse habitats and a strong adaptability

in changing environments (Imhoff, 2017; Nguyen et al., 2018; Khuong et al., 2023a). They have been widely applied for increasing crop yield, inducing stress resistance of plants, and heavy metal accumulation (Khan et al., 2023; Zeng et al., 2023). Therefore, they are a promising nutrition source for maize in a sustainable cultivation. Thus, the study was conducted to assess the effects of the four *Rhodopseudomonas palustris* strains on soil properties, growth and yield of hybrid maize cultivated on in-dyke alluvial soil.

## 2. Materials and methods

### 2.1. Materials

The greenhouse experiment was carried out in the period from February, 2020 to June, 2020. In-dyke alluvial soil was collected at sites bordered by dykes in Chau Phu district, An Giang province, Vietnam (10.535003, 105.273485). The soil parameters were characterized in Table 1.

**Table 1**  
Properties of initial soil for maize cultivation

Soil property	Value
pH <sub>Water</sub>	5.44 ± 0.07
pH <sub>KCl</sub>	4.76 ± 0.04
OM (% C)	1.32 ± 0.21
Total N (%)	0.17 ± 0.02
NH <sub>4</sub> <sup>+</sup> (mg NH <sub>4</sub> <sup>+</sup> kg <sup>-1</sup> )	38.2 ± 7.56
NO <sub>3</sub> <sup>-</sup> (mg NO <sub>3</sub> <sup>-</sup> kg <sup>-1</sup> )	21.3 ± 0.19
Total P (%)	0.036 ± 0.009
Available P (mg P kg <sup>-1</sup> )	38.6 ± 0.08

OM: Organic matter; N: Nitrogen, P: Phosphorus.

The alluvial soil was collected at the depth of 0–20 cm, dried out, and filtered from vegetative residues. Then, the soil was autoclaved 2 times at 121°C, 1 atm for 50 min each. The two times were separated for 24 h. On the other hand, the bacterial strains utilized in the current study were PNSB, *Rhodopseudomonas palustris*, including VNW64, VNS89, TLS06 and VNS02, possessing ability to fix N, solubilize P, and secrete plant growth promoting compounds (IAA, ALA and siderophores) and exopolymeric compounds (Khuong et al., 2017, 2020a). They were stored at –80°C in Faculty of Crop Science, College of Agriculture, Can Tho University. For the variety, hybrid maize CP 888 seeds utilized in the current study were provided by C.P. SEEDS Company, Dong Nai Province, Vietnam. The fertilizer utilized in the current study included Urea (46% N, Phu My Company, Ho Chi Minh City, Vietnam), super phosphate (16% P<sub>2</sub>O<sub>5</sub>, Long Thanh Company, Dong Nai Province, Vietnam), and KCl (60% K<sub>2</sub>O, Belarusian Potash Company, Minsk City, Belarus).

### 2.2. Methods

The pot experiment was designed following a completely randomized block with 10 treatments, 4 replicates for each treatment, and 1 pot for 1 replicate. The plastic pots had the size of pot top x small bottom x height of 25 x 21 x 21 (cm), respectively. Then, ten kg of soil was weighed and put into each pot.

The inorganic fertilizer formula utilized for hybrid maize in the Mekong Delta (kg ha<sup>-1</sup>) was 200 N – 90 P<sub>2</sub>O<sub>5</sub> – 80 K<sub>2</sub>O. The treatments were as follows: (i) 100% N (200 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>): fertilization with 100% N and 100% P of recommended fertilizer formula (RFF); (ii) 85% N (170 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>): fertilization with 85% and 100% P of RFF; (iii) 70% N (140 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>): fertilization with 70% N and 100% P of RFF; (iv) 55% N (110 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>): fertilization with 55% N and 100% P of RFF; (v) 100% N (200 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + PNSB: fertilization with 100% N and 100% P of RFF, combined with a mixture of the four PNSB (MFPNSB); (vi) 85% N (170 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + PNSB: fertilization with 85% N and 100% P of RFF, combined with MFPNSB; (vii) 70% N (140 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + PNSB: fertilization with 70% N and 100% P of RFF, combined with MFPNSB; (viii) 55% N (110 kg N ha<sup>-1</sup>) + 100% P (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + PNSB: fertilization with 55% N and 100% P of RFF, combined with MFPNSB; (ix) 0% N (0 kg N ha<sup>-1</sup>) + 0% P (0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>): no fertilization; and (x) 0% N (0 kg N ha<sup>-1</sup>) + 0% P (0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + PNSB: only MFPNSB. The rates of chemical fertilizers, e.g. 100%, 85%, 70%, 55%, and 0%, were the percentage of using the particular fertilizer according to the following inorganic fertilizer formula.

Based on the soil bulk density (1.0 g cm<sup>-3</sup>), cultivatable horizon depth (0.2 m), the total volume of soil was 2,000,000 kg soil ha<sup>-1</sup>. The amount of fertilizers for 10 kg soil was calculated 1 g N, 0.45 g P<sub>2</sub>O<sub>5</sub>, and 0.40 g K<sub>2</sub>O. In the current study, the fertilization was performed 4 times. In the first time: all of the P fertilizer was used before planting; in the second time 2: 30% of N and 50% of K were used at 10 days after planting (DAP); in the third time: 40% of N was used at 20 DAP; and in the fourth time: 30% of N and 50% of K were used at 45 DAP.

The greenhouse condition consisted of the temperature 37°C and humidity 62%, the light and dark hours per day were 10 and 14, respectively.

#### 2.2.1. Inoculation of bacteria on maize seeds

The bacterial density was calculated according to the Most Probable Number (MPN) method for PNSB (Harada et al., 2005). The preparation was according to the method used in the study by Thuc et al. (2022) with some modifications. In particular, the maize grains were sterilized with 70% ethanol and 1% sodium hypochlorite solution for 3 and 10 min, respectively. Then, they were rinsed twice with autoclaved distilled water. The grains were incubated for 24 h under dark conditions to germinate. The germinated maize seeds were placed in a beaker containing 200 mL of a mixed broth of *R. palustris* VNW64, VNS89, TLS06 and VNS02 with a density of 10<sup>9</sup> MPN mL<sup>-1</sup>. The mixture was shaken for 1 h at 120 rpm. In the negative control, autoclaved distilled water was used instead of the bacterial mixture. In each

seed per pot, the bacterial density was  $1.0 \times 10^6$  MPN  $g^{-1}$  dry soil. Fifteen mL of liquid bacteria was applied at 24, 32, and 61 DAP. Thus, the overall bacterial mixture applied to seeds and soil in a crop was  $1.1 \times 10^6$  MPN  $g^{-1}$  dry soil.

### 2.2.2. Agronomic parameters

Parameters were evaluated at 108 DAP as follows: Plant height (cm) was measured from the ground to the peak of a plant; Ear forming height (cm) was measured from the ground to when an ear with yield appeared; Stem diameter (cm) was measured by the average diameter derived from ground, middle and top of a plant; Leaves/plant (leaves) were calculated by counting all of the leaves appeared from the ground to the peak of a plant; Leaf length (cm) was measured from the top to petiole of the fifth leaf; and Leaf width (cm) was measured at the middle of the fifth leaf.

### 2.2.3. Yield components

The components were measured at 108 DAP as follows: Ear length (cm) was measured from both ends; Ear diameter (cm) was measured averagely at 3 positions, top, middle and crown; The number of rows per ear (row  $ear^{-1}$ ) was calculated by counting the number of rows per ear; and Number of seeds per row (seeds  $row^{-1}$ ) was calculated by counting the number of seeds in a row.

### 2.2.4. Maize yield ( $g\ plant^{-1}$ ) and dry biomass ( $g\ plant^{-1}$ )

Grain weight of each pot was weighed at 108 DAP, its humidity was measured and yield was calculated at 15.5% humidity. On the contrary, plant parts, including root, stem, leaves, sheath and core, were dried up at  $70^\circ C$  for 72 h.

### 2.2.5. Analysis of N and P in plant parts at harvest

The plant samples were dried up at  $70^\circ C$  for 72 h and ground through a 0.2 mm sieve. Then, 0.3 g of sample (proceeding separately from each parts of the plant) was put into 3.3 mL of a mixture of 18 mL diluted water, 100 mL saturated  $H_2SO_4$ , and 6 g salicylic acid. The mixture was put on a hotplate for 1 day, and a solution of 30%  $H_2O_2$  (3–4 drops for each) was added until the sample liquid was clear. Inorganic turned solution was scaled at a volume of 50 mL in order to determine the concentrations of N and P in the root, stem, leaves, seeds, outer sheath and core, following the method of Houba et al. (1997).

### 2.2.6. Calculation of N and P uptake of hybrid maize

The N uptake in a plant part was calculated as the multiplication of the N concentration and dry biomass of that part. The total N uptake was the sum of N uptake in root, stem, leaves, seeds, sheath and core. On the other hand, the P uptake shared the similar calculation.

### 2.2.7. Soil analysis

According to Sparks et al. (1996), analytic methods were described as follows:  $pH_{water}$  or  $pH_{KCl}$  was extracted by a ratio of soil: water (1: 5) or soil: KCl 1.0 M (1: 5), and measured by a pH meter. The extracted pH solution was utilized for measuring EC by an EC meter. Cation exchange capacity (CEC) was extracted

by 0.1 M  $BaCl_2$ , and titrated by 0.01 M EDTA. Total N content was turned into organic compounds by a mixture of saturated  $H_2SO_4$ :  $CuSO_4$ : Se, with a ratio of 100: 10: 1 and determined by Kjeldahl method. Available N in  $NH_4^+$  form was extracted by 2.0 M KCl, visualized in color by a mixture of sodium nitropruside, sodium salicylate, sodium citrate, sodium tartrate, sodium hydroxide and sodium hypochlorite, and measured at a wavelength of 650 nm. Organic matter was identified according to the Walkley–Black method: the soil was oxidized by saturated  $H_2SO_4 - K_2Cr_2O_7$  mixture and titrated by 0.5 N  $FeSO_4$ . The total P content in the soil was turned into inorganic forms by a mixture of saturated  $H_2SO_4 - HClO_4$ . The solution after that was scaled at a volume of 50 mL and then used to measure in color by ascorbic acid at a wavelength of 880 nm. Soluble P content was determined by Bray II method: it was extracted from soil by a mixture of 0.1 N HCl and 0.03 N  $NH_4F$ , with a ratio of soil: water of 1: 7. Then, it was measured by color comparison method at a wavelength of 880 nm. Insoluble P compounds including Fe-P, Al-P and Ca-P, were extracted by solutions, including 0.1 M NaOH, 0.5 M  $NH_4F$  and 0.25 M  $H_2SO_4$ , respectively. Then, they were analyzed in color following a method determining total P content. The soil properties at the crop beginning are shown in Table 1.

## 2.3. Statistical analysis

The input data were proceeded by Microsoft Excel 2019. The data were checked for normal distribution in advances for subjecting one-way ANOVA by SPSS software, version 13.0. Duncan's test was utilized for comparing differences of mean values among treatments at 5% level of significance.

## 3. Results

### 3.1. Effects of potent *Rhodopseudomonas palustris* strains on in-dyke soil fertility cultivated maize

The  $pH_{water}$  values in treatments inoculated with MFPNSB fluctuated from 6.11 to 6.15, significantly higher ( $p\ 0.00950 \times 10^{-5} < 0.05$ ) than those in treatments without supplemented bacteria, ranging from 5.74 to 5.99. However, among treatments supplemented with bacteria, results of  $pH_{KCl}$ , EC, CEC,  $N_{total}$ ,  $P_{total}$ , and organic matter had no statistical difference. In brief, the total concentration of N, P and organic matter ranged from 0.178 to 0.232%, from 0.053 to 0.064% and from 1.95 to 2.47%, respectively (Table 2).

The available N contents ( $NH_4^+$  form) varied significantly ( $p\ 0.0070 \times 10^{-6} < 0.05$ ) between treatments supplemented with MFPNSB and treatments without supplemented bacteria at the same N rate. In details, at harvest, treatments supplemented with MFPNSB had soil  $NH_4^+$  content ranging from 16.4–17.7 mg  $NH_4^+ kg^{-1}$ , while the content in treatments without supplemented bacteria ranged from 11.3–12.7 mg  $NH_4^+ kg^{-1}$ . Interestingly, the treatment fertilized with 0% N of RFF plus MFPNSB possessed an amount of soil  $NH_4^+$  of 16.7 mg  $NH_4^+ kg^{-1}$ , statistically equal to all other treatments (Table 2).

Table 2

Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on characteristics of in-dyke alluvial soil planting maize in greenhouse condition

Treatment	pH <sub>Water</sub>	pH <sub>KCl</sub>	N <sub>Total</sub>	P <sub>Total</sub>	N <sub>Available</sub>	P <sub>Available</sub>
	–	–	%	%	mg NH <sub>4</sub> <sup>+</sup> kg <sup>-1</sup>	mg P kg <sup>-1</sup>
100% N + 100% P	5.74 <sup>c</sup>	4.84	0.232	0.055	11.3 <sup>b</sup>	64.9 <sup>cd</sup>
85% N + 100% P	5.87 <sup>b</sup>	4.99	0.222	0.061	11.5 <sup>b</sup>	65.7 <sup>cd</sup>
70% N + 100% P	5.98 <sup>b</sup>	5.07	0.220	0.053	11.4 <sup>b</sup>	65.1 <sup>cd</sup>
55% N + 100% P	5.99 <sup>b</sup>	5.26	0.188	0.058	12.7 <sup>b</sup>	63.5 <sup>d</sup>
100% N + 100% P + MFPNSB	6.11 <sup>a</sup>	5.12	0.283	0.060	17.7 <sup>a</sup>	73.6 <sup>a</sup>
85% N + 100% P + MFPNSB	6.12 <sup>a</sup>	5.01	0.242	0.055	16.8 <sup>a</sup>	71.6 <sup>ab</sup>
70% N + 100% P + MFPNSB	6.15 <sup>a</sup>	5.05	0.230	0.059	16.4 <sup>a</sup>	68.6 <sup>bc</sup>
55% N + 100% P + MFPNSB	6.12 <sup>a</sup>	4.99	0.222	0.064	16.9 <sup>a</sup>	68.4 <sup>bc</sup>
0% N + 0% P	5.94 <sup>b</sup>	5.14	0.178	0.058	11.9 <sup>b</sup>	57.7 <sup>e</sup>
0% N + 0% P + MFPNSB	6.11 <sup>a</sup>	4.94	0.192	0.055	16.7 <sup>a</sup>	63.7 <sup>d</sup>
Significant difference	*	ns	ns	ns	*	*
C.V. (%)	1.28	4.10	4.20	5.60	9.36	4.06

Treatment	EC	CEC	OM	Al-P	Ca-P	Fe-P
	mS cm <sup>-1</sup>	meq 100 g <sup>-1</sup>	%C	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>
100% N + 100% P	0.34	16.9	2.12	64.8 <sup>a</sup>	157.8 <sup>abc</sup>	171.3 <sup>a</sup>
85% N + 100% P	0.27	17.8	2.19	67.4 <sup>a</sup>	167.3 <sup>a</sup>	138.0 <sup>b</sup>
70% N + 100% P	0.24	16.3	2.47	65.1 <sup>a</sup>	158.7 <sup>ab</sup>	141.8 <sup>b</sup>
55% N + 100% P	0.26	16.2	2.22	66.1 <sup>a</sup>	171.9 <sup>a</sup>	137.9 <sup>b</sup>
100% N + 100% P + MFPNSB	0.29	15.1	2.12	58.1 <sup>b</sup>	132.4 <sup>de</sup>	123.1 <sup>c</sup>
85% N + 100% P + MFPNSB	0.28	15.4	2.42	55.6 <sup>bc</sup>	138.5 <sup>d</sup>	123.2 <sup>c</sup>
70% N + 100% P + MFPNSB	0.23	16.9	1.97	54.3 <sup>c</sup>	143.5 <sup>cd</sup>	114.2 <sup>cd</sup>
55% N + 100% P + MFPNSB	0.26	16.4	1.95	53.9 <sup>cd</sup>	146.3 <sup>bcd</sup>	108.5 <sup>d</sup>
0% N + 0% P	0.21	15.7	1.97	66.0 <sup>a</sup>	143.8 <sup>cd</sup>	89.7 <sup>e</sup>
0% N + 0% P + MFPNSB	0.25	17.5	2.19	51.5 <sup>d</sup>	123.4 <sup>e</sup>	79.4 <sup>f</sup>
Significant difference	ns	ns	ns	*	*	*
C.V. (%)	27.1	11.3	22.7	2.85	6.37	5.30

Numbers in each column with the same following letters are not significantly different from each other. ns: not significant difference; \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus; EC: Electrical conductivity, CEC: Cation exchangeable capacity, OM: Organic matter.

At the same N rate, except for at 70% N of RFF, treatments without supplemented MFPNSB strains had soluble P contents ranging from 57.7 to 65.7 mg P kg<sup>-1</sup>, significantly lower than treatments with supplemented MFPNSB. Additionally, the treatment supplemented with MFPNSB, but no N fertilizer, provided a higher soluble soil P content (63.7 mg P kg<sup>-1</sup>) than the treatment fertilized with no fertilizer (57.7 mg P kg<sup>-1</sup>). Moreover, it was equivalent to the contents in treatments without supplemented bacteria plus N fertilizer at different rates (Table 2).

Insoluble P contents, including Al-P, Ca-P, and Fe-P, in treatments without supplemented MFPNSB, were higher than those in treatments fertilized with MFPNSB (Table 2). To be more specific, the treatment fertilized with 100% N of RFF had Al-P, Ca-P, and Fe-P amounts of 64.8, 157.8 and 171.3 mg P kg<sup>-1</sup>, respectively, significantly higher ( $p 0.0012 \times 10^{-12}$ ,  $0.0079 \times 10^{-4}$ ,  $0.0032 \times 10^{-13}$ ,

$< 0.05$ , respectively) than the treatment fertilized with 100% N of RFF plus MFPNSB where the values were 58.1, 132.4 and 123.1 mg P kg<sup>-1</sup>, in the same order. In treatments fertilized with 85, 70, 55 and 0% N of RFF plus MFPNSB, the amount of Al-P and Fe-P in soil was remarkably reduced after a crop of maize (Table 2).

### 3.2. Effects of potent *Rhodopseudomonas palustris* strains on nitrogen uptake of maize cultivated in in-dyke alluvial soil

#### 3.2.1. Nitrogen concentration in hybrid maize parts

In root, at a N rate, except for at 55% N of RFF, the treatment supplemented with MFPNSB possessed a significantly different N concentration ( $p 0.0040 \times 10^{-12} < 0.05$ ) from that of the treatment without supplemented bacteria. Following the order of N

rates 70–100, 55, and 0% N of RFF, N concentrations in root were roughly 0.66–0.92, 0.65 and 0.47% in treatments without supplemented bacteria, and 1.06–1.22, 0.74 and 0.68% in treatments supplied with MFPNSB, respectively (Table 3).

In stem, only in the bacteria applied treatments fertilized with 100 and 55% N of RFF, the N concentrations were higher than that in the treatments without supplemented bacteria, with 1.38 and 0.76% compared to 0.89 and 0.59%, respectively. Additionally, in seeds, at N rates of 100 and 0% N of RFF, N concentrations in the treatments supplemented with MFPNSB were 1.07% and 0.58%, respectively, significantly higher than the treatments without supplemented MFPNSB with nitrogen concentrations of 0.89 and 0.43%, respectively (Table 3).

In leaves, sheath and core, at all N rates, the N concentrations in treatments supplemented with MFPNSB were higher than those in the treatments without supplemented bacteria. In

details, in leaves, sheath and core, for treatments supplemented with bacteria, the N concentrations ranged 0.47–0.81, 0.39–0.98, and 0.37–1.11%, in comparison with 0.70–1.02, 0.62–1.14 and 0.49–1.24% in the treatments without supplemented bacteria, respectively (Table 3).

### 3.2.2. Dry biomass in hybrid maize parts

In root, for treatments fertilized with 100, 70 and 55% N of RFF plus MFPNSB, the biomass were higher, significantly different ( $p < 0.0097 \times 10^{-13} < 0.05$ ), than treatments fertilized with the same N rates of RFF but no bacteria applied, with 6.82–8.25 g pot<sup>-1</sup> in comparison with 5.30–7.51 g pot<sup>-1</sup>, respectively. Nevertheless, at the rate of 85% N of RFF, the biomass difference between treatments with and without supplemented bacteria was similar. When there was no chemical fertilizer applied, the biomass of dry root was remarkably higher in the treatment

**Table 3**

Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on dry biomass of parts of maize planted in in-dyke alluvial soil in greenhouse condition

Treatment	Biomass (g pot <sup>-1</sup> )						N content (%)		
	Root	Stem	Leaf	Seed	Outer sheath	Core	Root	Stem	Leaf
100% N + 100% P	7.51 <sup>b</sup>	148.9 <sup>b</sup>	36.8 <sup>c</sup>	100.6 <sup>b</sup>	20.8 <sup>b</sup>	28.6 <sup>cd</sup>	0.92 <sup>c</sup>	0.89 <sup>b</sup>	0.81 <sup>b</sup>
85% N + 100% P	7.16 <sup>bc</sup>	127.7 <sup>c</sup>	34.8 <sup>d</sup>	98.3 <sup>b</sup>	18.0 <sup>cd</sup>	28.3 <sup>d</sup>	0.82 <sup>d</sup>	0.74 <sup>b</sup>	0.81 <sup>b</sup>
70% N + 100% P	6.44 <sup>d</sup>	104.1 <sup>e</sup>	34.1 <sup>d</sup>	95.2 <sup>c</sup>	17.3 <sup>cde</sup>	28.0 <sup>d</sup>	0.66 <sup>e</sup>	0.76 <sup>b</sup>	0.68 <sup>c</sup>
55% N + 100% P	5.30 <sup>e</sup>	86.7 <sup>e</sup>	33.6 <sup>d</sup>	84.9 <sup>e</sup>	17.1 <sup>de</sup>	28.3 <sup>d</sup>	0.65 <sup>e</sup>	0.59 <sup>c</sup>	0.61 <sup>c</sup>
100% N + 100% P + MFPNSB	8.25 <sup>a</sup>	194.1 <sup>a</sup>	43.0 <sup>a</sup>	110.1 <sup>a</sup>	22.9 <sup>a</sup>	31.2 <sup>ab</sup>	1.22 <sup>a</sup>	1.38 <sup>a</sup>	0.98 <sup>a</sup>
85% N + 100% P + MFPNSB	7.42 <sup>b</sup>	151.1 <sup>b</sup>	38.9 <sup>b</sup>	109.2 <sup>a</sup>	22.0 <sup>a</sup>	31.8 <sup>a</sup>	1.10 <sup>b</sup>	0.82 <sup>b</sup>	0.99 <sup>a</sup>
70% N + 100% P + MFPNSB	7.05 <sup>bc</sup>	115.3 <sup>d</sup>	37.6 <sup>bc</sup>	98.7 <sup>b</sup>	20.5 <sup>b</sup>	29.9 <sup>bc</sup>	1.06 <sup>b</sup>	0.79 <sup>b</sup>	1.02 <sup>a</sup>
55% N + 100% P + MFPNSB	6.82 <sup>cd</sup>	103.1 <sup>e</sup>	37.1 <sup>bc</sup>	90.2 <sup>d</sup>	18.4 <sup>c</sup>	30.1 <sup>b</sup>	0.74 <sup>de</sup>	0.76 <sup>b</sup>	0.82 <sup>b</sup>
0% N + 0% P	4.38 <sup>f</sup>	53.9 <sup>h</sup>	33.9 <sup>d</sup>	74.5 <sup>f</sup>	12.7 <sup>f</sup>	20.6 <sup>f</sup>	0.47 <sup>f</sup>	0.36 <sup>d</sup>	0.47 <sup>d</sup>
0% N + 0% P + MFPNSB	4.95 <sup>e</sup>	92.7 <sup>f</sup>	37.4 <sup>bc</sup>	83.9 <sup>e</sup>	16.2 <sup>e</sup>	24.2 <sup>e</sup>	0.68 <sup>e</sup>	0.48 <sup>cd</sup>	0.70 <sup>bc</sup>
Significant difference	*	*	*	*	*	*	*	*	*
C.V. (%)	4.87	3.20	3.35	2.08	4.27	3.55	7.62	12.55	9.85

Treatment	N uptake (g pot <sup>-1</sup> )						N content (%)		
	Root	Stem	Leaf	Seed	Outer sheath	Core	Seed	Outer sheath	Core
100% N + 100% P	0.069 <sup>c</sup>	1.318 <sup>b</sup>	0.295 <sup>b</sup>	0.903 <sup>c</sup>	0.205 <sup>bc</sup>	0.318 <sup>b</sup>	0.89 <sup>bc</sup>	0.98 <sup>b</sup>	1.11 <sup>b</sup>
85% N + 100% P	0.058 <sup>d</sup>	0.937 <sup>c</sup>	0.280 <sup>b</sup>	0.855 <sup>c</sup>	0.152 <sup>d</sup>	0.248 <sup>c</sup>	0.87 <sup>bc</sup>	0.83 <sup>c</sup>	0.87 <sup>c</sup>
70% N + 100% P	0.042 <sup>ef</sup>	0.787 <sup>c</sup>	0.230 <sup>d</sup>	0.800 <sup>c</sup>	0.130 <sup>ef</sup>	0.153 <sup>d</sup>	0.84 <sup>c</sup>	0.76 <sup>c</sup>	0.55 <sup>e</sup>
55% N + 100% P	0.035 <sup>fg</sup>	0.517 <sup>d</sup>	0.202 <sup>de</sup>	0.542 <sup>e</sup>	0.115 <sup>fg</sup>	0.123 <sup>e</sup>	0.64 <sup>de</sup>	0.67 <sup>d</sup>	0.42 <sup>fg</sup>
100% N + 100% P + MFPNSB	0.100 <sup>a</sup>	2.667 <sup>a</sup>	0.422 <sup>a</sup>	1.180 <sup>a</sup>	0.262 <sup>a</sup>	0.385 <sup>a</sup>	1.07 <sup>a</sup>	1.14 <sup>a</sup>	1.24 <sup>a</sup>
85% N + 100% P + MFPNSB	0.082 <sup>b</sup>	1.232 <sup>b</sup>	0.387 <sup>a</sup>	1.045 <sup>b</sup>	0.220 <sup>b</sup>	0.375 <sup>a</sup>	0.96 <sup>b</sup>	0.99 <sup>b</sup>	1.18 <sup>ab</sup>
70% N + 100% P + MFPNSB	0.075 <sup>bc</sup>	0.912 <sup>c</sup>	0.380 <sup>a</sup>	0.828 <sup>c</sup>	0.192 <sup>c</sup>	0.222 <sup>c</sup>	0.84 <sup>c</sup>	0.94 <sup>b</sup>	0.75 <sup>d</sup>
55% N + 100% P + MFPNSB	0.050 <sup>e</sup>	0.777 <sup>c</sup>	0.305 <sup>b</sup>	0.652 <sup>d</sup>	0.140 <sup>de</sup>	0.167 <sup>d</sup>	0.73 <sup>d</sup>	0.77 <sup>c</sup>	0.55 <sup>e</sup>
0% N + 0% P	0.020 <sup>h</sup>	0.192 <sup>e</sup>	0.157 <sup>e</sup>	0.320 <sup>f</sup>	0.047 <sup>h</sup>	0.078 <sup>f</sup>	0.43 <sup>f</sup>	0.39 <sup>e</sup>	0.37 <sup>g</sup>
0% N + 0% P + MFPNSB	0.034 <sup>g</sup>	0.445 <sup>d</sup>	0.263 <sup>bc</sup>	0.482 <sup>e</sup>	0.100 <sup>g</sup>	0.117 <sup>e</sup>	0.58 <sup>e</sup>	0.62 <sup>d</sup>	0.49 <sup>ef</sup>
Significant difference	*	*	*	*	*	*	*	*	*
C.V. (%)	9.81	12.90	10.83	9.30	8.12	9.20	9.01	6.78	8.42

Numbers in each column with the same following letters are not significantly different from each other. \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus

supplemented with MFPNSB (4.95 g pot<sup>-1</sup>) than in the treatment without supplemented bacteria (3.38 g pot<sup>-1</sup>) (Table 3).

In stem, leaves, seeds, sheath and core, at same N rates of 100, 85, 70, 55, and 0% N of RFF, biomass in treatments supplemented with MFPNSB were higher than those in the treatment without supplemented bacteria, with 194.1, 151.1, 115.3, 103.1, and 92.7 g pot<sup>-1</sup> compared to 148.9, 127.7, 104.1, 87.6, and 53.9 g pot<sup>-1</sup>, respectively, for dry stem biomass; 33.6–36.8 compared to 37.1–43.0 g pot<sup>-1</sup> for dry leaves biomass; 74.5–100.6 compared to 83.9–110.1 g pot<sup>-1</sup> for dry seeds biomass; 12.7–20.8 compared to 16.2–22.9 g pot<sup>-1</sup> for dry sheath biomass; and 20.6–28.6 compared to 24.2–31.2 g pot<sup>-1</sup> for dry core biomass. Moreover, in the case using 55–85% N of RFF, treatments combined with MFPNSB had biomass statistically equal to those with 15% N higher in dry stem, leaves, seeds, sheath and core. Surprisingly, the biomass of dry stem and leaves in the treatment receiving no N fertilizer but supplemented MFPNSB (92.7 and 37.4 g pot<sup>-1</sup>, respectively) was even equivalent to that in the treatment fertilized with 55% N of RFF (86.7 and 33.6 g pot<sup>-1</sup>, respectively). Meanwhile, in the dry core, the biomass in the treatment fertilized with 0% N of RFF plus MFPNSB (24.2 g pot<sup>-1</sup>) was lower than that in the treatment fertilized with 55% N of RFF (28.3 g pot<sup>-1</sup>). At the same time, the biomass of the dry root, seeds and sheath in the treatment supplemented with only MFPNSB was equal to that in the treatment with 55% N of RFF (Table 3).

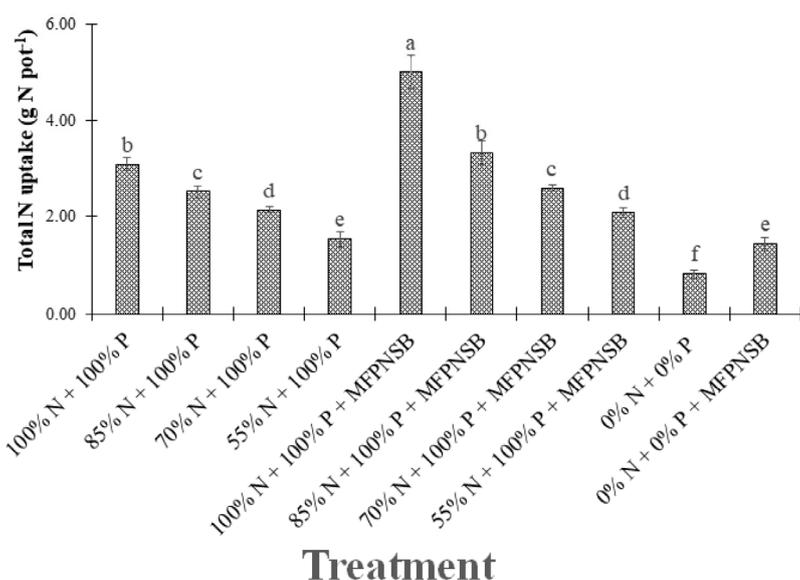
### 3.2.3. Nitrogen uptake in parts of hybrid maize

A decline in N fertilizer rates led to a reduction in N uptake in plant parts. A decrease from 100% N to 55% N of RFF resulted in drops in N uptake from 0.069 to 0.035 g N pot<sup>-1</sup> in root, from 1.318 to 0.517 g N pot<sup>-1</sup> in stem, from 0.295 to 0.202 g N pot<sup>-1</sup> in leaves, from 0.903 to 0.542 g N pot<sup>-1</sup> in seeds, from 0.205 to 0.115 g N pot<sup>-1</sup> in sheath and from 0.318 to 0.123 g N pot<sup>-1</sup> in core. At the same N fertilizer rates, treatments supplemented with MFPNSB shared the same trend, but had the better amount of N uptake, in comparison with the treatments without supplemented bacteria, except for in stem and seeds at N fertilizer rate

of 70% N of RFF. The treatments supplemented with MFPNSB possessed dominant N uptake, in comparison with the treatments without supplemented bacteria. Following the reduction of N fertilizer rates of 100, 85, 70, 55, and 0% N of RFF, the values were 0.100, 0.082, 0.075, 0.050, and 0.034 g N pot<sup>-1</sup> compared to 0.069, 0.058, 0.042, 0.035, and 0.020 g N pot<sup>-1</sup> in root; 0.422, 0.387, 0.380, 0.305, and 0.263 g N pot<sup>-1</sup> compared to 0.295, 0.280, 0.230, 0.202, and 0.157 g N pot<sup>-1</sup> in leaves; 1.18, 1.045, 0.828, 0.652, and 0.482 g N pot<sup>-1</sup> compared to 0.903, 0.855, 0.800, 0.542 and 0.320 g N pot<sup>-1</sup> in seeds; 0.262, 0.220, 0.192, 0.140, and 0.100 g N pot<sup>-1</sup> compared to 0.205, 0.152, 0.130, 0.115 and 0.047 g N pot<sup>-1</sup> in sheath; and 0.385, 0.375, 0.222, 0.167, and 0.117 g N pot<sup>-1</sup> compared to 0.318, 0.248, 0.153, 0.123, and 0.078 g N pot<sup>-1</sup> in core, respectively. These results indicated that the supplementation of MFPNSB raised the N uptake in the root, stem, leaves, seeds, sheath and core. Moreover, in the treatment that used only MFPNSB, the value was even equivalent to that in the treatment fertilized with 55% N of RFF. Furthermore, the result in Table 3 showed that in the treatments fertilized with 100 and 85% N of RFF plus MFPNSB, the N uptake ranked as follows: stem > seeds > leaves > core > sheath > roots.

### 3.2.4. Total nitrogen uptake in hybrid maize

Reducing the amount of N fertilizer used resulted in a decline in the total N uptake of hybrid maize. The uptake were 3.11, 2.53, 2.15 and 1.53 g N pot<sup>-1</sup>, corresponding to N fertilizer rates of 100, 80, 75, 55 and 0% N of RFF. The treatment fertilized with 85% N of RFF plus MFPNSB had a total N uptake of 3.34 g N pot<sup>-1</sup>, statistically equal to that in the treatment fertilized with 100% N of RFF, but significantly lower than the uptake in the treatment fertilized with 100% N plus MFPNSB (5.03 g N pot<sup>-1</sup>). The treatment fertilized with no fertilization and without supplemented bacteria had the smallest N uptake (0.82 g N pot<sup>-1</sup>). Nevertheless, in the treatment supplemented with only MFPNSB, the total N uptake was 1.43 g N pot<sup>-1</sup>, equal to the uptake in the treatment fertilized with 55% N of RFF (1.53 g N pot<sup>-1</sup>) (Fig. 1).



**Fig. 1.** Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on total nitrogen uptake of maize planted in in-dyke alluvial soil in greenhouse condition. The bars with same following letters are not significantly different from each other. ns: not significant difference; \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus; EC: Electrical conductivity, CEC: Cation exchangeable capacity, OM: Organic matter, MFPNSB: a mixture of the four purple nonsulfur bacteria strains

### 3.3. Effects of potent *Rhodopseudomonas palustris* strains on growth, yield components and yield of maize cultivated in in-dyke alluvial soil

#### 3.3.1. Growth of hybrid maize

Plants in treatment fertilized with 100 N of RFF plus MFPNSB, where the height was 207.5 cm, were significantly higher ( $p < 0.0084 \times 10^{-19} < 0.05$ ) than all of the other treatments. Plant height in the treatment fertilized with 70% N of RFF plus MFPNSB was 180.5 cm, 3.0 cm taller than that in the treatment only used 70% N of RFF (177.5 cm). The treatment fertilized with 85% N of RFF plus MFPNSB had a plant height of 187.8 cm, where the plant was 5.3 cm taller than in the treatment fertilized with the same N rate but no applied bacteria. Additionally, the treatment fertilized with 85% N of RFF plus MFPNSB had an equal average plant height to the treatment of 100% N of RFF (186.8 cm). Moreover, the treatment supplemented with only MFPNSB resulted in plants with a height of 160.8 cm, while the treatment fertilized without any fertilizers resulted in plants with a height of 140.3 cm (Table 4). Furthermore, in the treatment supplemented with only MFPNSB, the height of appearing ear was 70.3 cm, significantly higher than that in the treatment without applied bacteria and N fertilizer (64.7 cm). However, only at N fertilizer rates of 100% N and 55%N plus MFPNSB, the height of appearing ear differed significantly ( $p < 0.0015 \times 10^{-19} < 0.05$ ), in comparison with treatments fertilized with only chemical fertilizers, with 89.3 and 120.9 cm compared to 81.7 and 99.8, respectively (Table 4).

Stem diameter of hybrid maize in the treatments fertilized with N fertilizer rates of 100, 85, 70 and 55% N of RFF plus MF-

PNSB were recorded as 2.14, 1.84, 1.74 and 1.71 cm, significantly higher than those in the treatments at the same N fertilizer rates but no bacteria applied, with 1.90, 1.60, 1.55 and 1.46 cm, respectively. The treatment fertilized with 85% N of RFF plus MFPNSB had an average stem diameter equal to that of the treatment fertilized with 100% N of RFF (Table 4).

Leaf size, in the treatment fertilized with 100% N of RFF plus MFPNSB was the biggest. Leaf size in the treatments supplemented with MFPNSB and treatments without supplemented bacteria ranged from 55.6 to 63.2 cm and from 54.9 to 58.2 cm in length, and from 5.73 to 7.65 and from 5.15 to 6.75 cm in width, respectively (Table 4).

#### 3.3.2. Yield components of hybrid maize

The treatment fertilized with 85% N of RFF plus MFPNSB had values of ear length and seeds number per row of 18.2 cm and 43.8 seeds row<sup>-1</sup>, both higher than those in the treatment fertilized with 100% N of RFF plus MFPNSB, with 17.4 cm and 40.3 seeds row<sup>-1</sup>. In addition, the treatment fertilized with 55% N of RFF plus MFPNSB and the treatment fertilized with 100% N of RFF had insignificant differences in ear diameter, and number of rows per ear. However, the treatment fertilized with 55% N of RFF plus MFPNSB was higher than the treatment fertilized with 100% of RFF in the values of ear length and number of seeds per row. Moreover, the treatment fertilized without both bacteria and inorganic fertilizers had the lowest values of ear diameter and number of rows per ear, while these values in the treatment fertilized with only MFPNSB were 3.21 cm and 10.5 rows ear<sup>-1</sup>, equivalent to the parameters in the treatment supplemented with 70% N of RFF (Table 5).

**Table 4**

Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on growth of maize planted in in-dyke alluvial soil in greenhouse condition

Treatment	Plant height	Height of appeared ear	Stem diameter	Numbers of leaf	Leaf length	Leaf width
	cm	cm	cm	leaf	cm	cm
100% N + 100% P	186.8 <sup>b</sup>	99.8 <sup>b</sup>	1.90 <sup>b</sup>	15.8 <sup>bc</sup>	58.2 <sup>bc</sup>	6.75 <sup>b</sup>
85% N + 100% P	182.3 <sup>c</sup>	91.6 <sup>cd</sup>	1.60 <sup>ef</sup>	16.3 <sup>ab</sup>	56.9 <sup>cd</sup>	5.95 <sup>c</sup>
70% N + 100% P	177.5 <sup>d</sup>	89.4 <sup>d</sup>	1.55 <sup>fg</sup>	15.8 <sup>bc</sup>	56.3 <sup>de</sup>	5.90 <sup>c</sup>
55% N + 100% P	171.5 <sup>e</sup>	81.7 <sup>e</sup>	1.46 <sup>g</sup>	16.3 <sup>ab</sup>	55.5 <sup>de</sup>	5.88 <sup>c</sup>
100% N + 100% P + MFPNSB	207.5 <sup>a</sup>	120.9 <sup>a</sup>	2.14 <sup>a</sup>	16.8 <sup>a</sup>	63.2 <sup>a</sup>	7.65 <sup>a</sup>
85% N + 100% P + MFPNSB	187.8 <sup>b</sup>	94.2 <sup>c</sup>	1.84 <sup>bc</sup>	16.0 <sup>ab</sup>	58.3 <sup>bc</sup>	6.77 <sup>b</sup>
70% N + 100% P + MFPNSB	180.5 <sup>cd</sup>	91.1 <sup>cd</sup>	1.74 <sup>cd</sup>	15.8 <sup>bc</sup>	58.8 <sup>b</sup>	5.90 <sup>c</sup>
55% N + 100% P + MFPNSB	177.5 <sup>d</sup>	89.3 <sup>d</sup>	1.71 <sup>de</sup>	16.0 <sup>ab</sup>	56.5 <sup>de</sup>	5.85 <sup>c</sup>
0% N + 0% P	140.3 <sup>g</sup>	64.7 <sup>g</sup>	1.08 <sup>h</sup>	14.3 <sup>d</sup>	54.9 <sup>e</sup>	5.15 <sup>d</sup>
0% N + 0% P + MFPNSB	160.8 <sup>f</sup>	70.3 <sup>f</sup>	1.16 <sup>h</sup>	15.0 <sup>cd</sup>	55.6 <sup>de</sup>	5.73 <sup>cd</sup>
Significant difference	*	*	*	*	*	*
C.V. (%)	1.62	2.63	4.79	3.49	1.83	6.50

Numbers in each column with the same following letters are not significantly different from each other. \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus.

Table 5

Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on yield components of maize planted in in-dyke alluvial soil in greenhouse condition

Treatment	Ear length	Ear diameter	Number of rows per ear	Number of seed per row
	cm	cm	row	seed
100% N + 100% P	17.4 <sup>c</sup>	3.20 <sup>bc</sup>	12.0 <sup>ab</sup>	40.3 <sup>c</sup>
85% N + 100% P	17.1 <sup>c</sup>	3.21 <sup>bc</sup>	11.0 <sup>ab</sup>	39.0 <sup>cd</sup>
70% N + 100% P	17.2 <sup>c</sup>	3.15 <sup>bc</sup>	10.5 <sup>bc</sup>	38.3 <sup>d</sup>
55% N + 100% P	17.1 <sup>c</sup>	3.08 <sup>c</sup>	12.0 <sup>ab</sup>	38.5 <sup>d</sup>
100% N + 100% P + MFPNSB	18.8 <sup>a</sup>	3.41 <sup>a</sup>	12.5 <sup>a</sup>	43.8 <sup>a</sup>
85% N + 100% P + MFPNSB	18.2 <sup>ab</sup>	3.30 <sup>ab</sup>	12.0 <sup>ab</sup>	43.8 <sup>a</sup>
70% N + 100% P + MFPNSB	18.1 <sup>b</sup>	3.31 <sup>ab</sup>	10.5 <sup>bc</sup>	39.3 <sup>cd</sup>
55% N + 100% P + MFPNSB	18.1 <sup>b</sup>	3.20 <sup>bc</sup>	11.5 <sup>ab</sup>	42.0 <sup>b</sup>
0% N + 0% P	12.6 <sup>e</sup>	2.11 <sup>d</sup>	9.00 <sup>c</sup>	23.3 <sup>f</sup>
0% N + 0% P + MFPNSB	14.8 <sup>d</sup>	3.17 <sup>bc</sup>	10.5 <sup>bc</sup>	27.5 <sup>e</sup>
Significant difference	*	*	*	*
C.V. (%)	2.20	3.05	10.63	2.61

Numbers in each column with the same following letters are not significantly different from each other. \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus.

### 3.3.3. Grain yield of hybrid maize

In comparison with the treatment fertilized with 100% N of RFF, the treatments fertilized with a decline by 15% N of RFF had lower grain yield significantly ( $p = 0.0016 \times 10^{-16} < 0.05$ ) (Fig. 2). The treatment fertilized with 100% N of RFF had grain yield of 83.0 g pot<sup>-1</sup>, while the others treatments, including those fertilized with 85, 70, and 55% N of RFF, resulted in 77.0, 72.6, and 66.1 g pot<sup>-1</sup>, respectively. In the treatment fertilized with 85% N

of RFF plus MFPNSB, grain yield was 93.4 g pot<sup>-1</sup>, while in the treatment fertilized with only 85% N of RFF, the value was only 77.0 g pot<sup>-1</sup>. Moreover, the treatment fertilized with 85% N of RFF plus MFPNSB had a better contribution to grain yield than the treatment fertilized with 100% N of RFF (83.0 g pot<sup>-1</sup>). The treatment fertilized with 55% N of RFF plus MFPNSB resulted in grain yield of 79.2 g pot<sup>-1</sup>, significantly higher than that of the treatment fertilized with the same N fertilizer rate, but bacteria

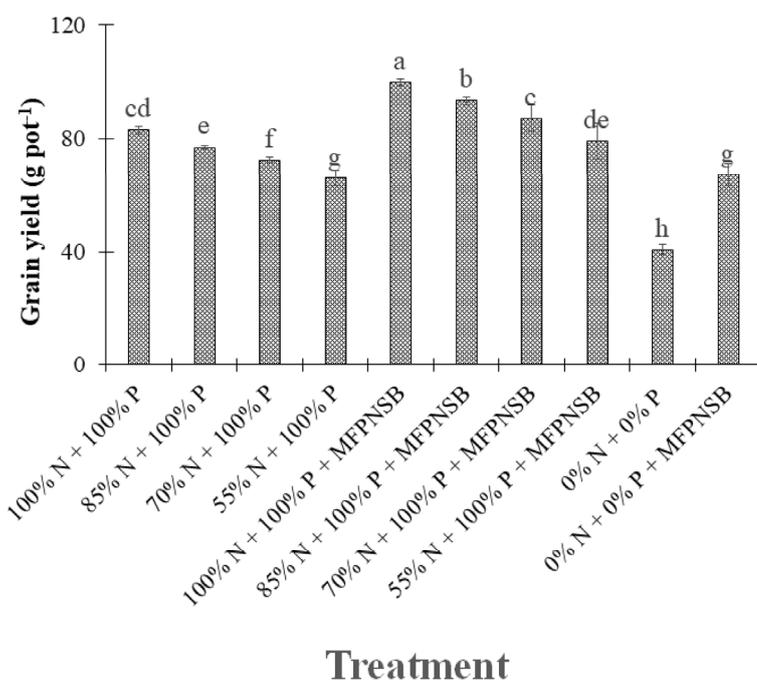


Fig. 2. Effects of potent *Rhodopseudomonas palustris* strains and nitrogen fertilizer rates on grain yield of maize planted in in-dyke alluvial soil in greenhouse condition. The bars with the same following letters are not significantly different from each other. ns: not significant difference; \*: significant difference ( $p < 0.05$ ) according to Duncan's test; MFPNSB: Mixture of *R. palustris* VNW64, VNS89, TLS06 and VNS02, N: Nitrogen, P: Phosphorus; EC: Electrical conductivity, CEC: Cation exchangeable capacity, OM: Organic matter, MFPNSB: a mixture of the four purple nonsulfur bacteria strains.

applied ( $66.1 \text{ g pot}^{-1}$ ) and equal to grain yield in the treatment fertilized with 70% N of RFF ( $72.6 \text{ g pot}^{-1}$ ). In particular, in the case of no inorganic fertilizer applied, the treatment supplemented with MFPNSB had higher grain yield than the treatment without supplemented bacteria, with 67.2 in comparison with  $40.8 \text{ g pot}^{-1}$ , respectively.

#### 4. Discussion

For the soil at the beginning of the crop, the mean of the total N content was 0.17% (Table 1), which is considered to be low threshold according to the classification of Metson (1961). Therefore, the input of biological nutrients can be seen as potential way to enhance maize yield sustainably. The result in Table 2 indicated that treatments supplemented with MFPNSB of *R. palustris* VNW64, VNS89, TLS06 and VNS02 had  $\text{pH}_{\text{Water}}$  equal or higher than 6.11, which is evaluated to be as slightly acidic soil. Meanwhile, in the treatments without supplemented bacteria, pH values were all lower than 6.00, considered as moderate acidity (Burt, 2014). This revealed that the treatments fertilized with both inorganic fertilizer and MFPNSB contributed to raising  $\text{pH}_{\text{Water}}$  value in in-dyke alluvial soil utilized for hybrid maize cultivation. Additionally, nutrients, including N and P, are essential and available for plants at soil pH roughly higher than 6.0 (Neina, 2019; Penn and Camberato, 2019). Thus, the treatments fertilized with MFPNSB mixture always had  $\text{pH}_{\text{Water}}$  values higher than or equal to 6.11, leading to higher availability of nutrients, and better nutrition uptake of plants. The result agreed with the study by Khuong et al. (2018), who stated that inoculation of PNSB strains on seeds and soil helps in enhancing alkaline soil pH for rice cultivation, due to the ability of these bacterial strains to produce ALA and EPS. Furthermore, bacterial strains of *R. palustris* VNW64, VNS89, TLS06, and VNS02 are capable of N fixing from air and P solubilizing from insoluble P compounds, including Al-P, Ca-P, and Fe-P (Khuong et al., 2017; 2022a; 2022c). Therefore, the treatments supplemented with MFPNSB had tremendously higher amounts of available N and soluble P contents, in comparison with the amounts that the treatments without applied bacteria had (Table 2). By solubilizing P compounds, the proportion of insoluble P, including Al-P, Ca-P, and Fe-P was lower in the treatment supplemented with MFPNSB, compared to treatments without supplemented MFPNSB mixture (Table 2). In the current study, the first eight treatments were applied with the same 100% P fertilizer rate to investigate the effects of the N fertilizers and the MFPNSB, while the other two treatments did not contain both N and P fertilizers so as to investigate the individual effects of the bacteria. However, in our future studies, a range of P fertilizer rates will be investigated.

As demonstrated by Mitra (2015), N concentration accounts for 1 – 6% of dry weight of plants, and plants take N in two forms,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The result of N concentration of the stem, leaves and sheath went up along with the increase of N fertilizer rates from 0 to  $200 \text{ kg ha}^{-1}$  (Table 3). Simultaneously, applying a mixture of *R. palustris* VNW64, VNS89, TLS06, and VNS02 improved the soil  $\text{NH}_4^+$  content (Table 2), and enhanced

N uptake in plants, leading to higher N uptake in plant parts in the treatments supplemented with MFPNSB than in the treatments without supplemented bacteria (Table 3). The result is consistent with previous studies, where using N-fixing *R. palustris* YSC3, YSC4, and PS3 boosted N uptake of Chinese cabbage (*Brassica rapa chinensis*) under low nutrition condition (Wong et al., 2014). In addition, bacterial strains of *R. palustris* are able to solubilize the insoluble P forms in order to provide soluble P to plants (Khuong et al., 2018, 2020a, 2022c), resulting in an increase in P concentration and uptake content in plants (Tables S1 and S2). The result was in accordance with the study by Rana et al. (2016), who demonstrated that supplying bacteria increased the solubilization of insoluble P compounds in soil, such as  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{Mg}_3(\text{PO}_4)_2$ , and  $\text{Zn}_3(\text{PO}_4)_2$ , resulting in P uptake in plants (Rafique et al., 2017). Apart from N-fixing and P-solubilizing abilities, PNSB strains can provide plant growth promoting substances, such as IAA and ALA (Khuong et al., 2018), stimulating plant growth by elongation of root tissues and better capacity in nutrients absorbance (Khuong et al., 2020a).

Reducing the N fertilizer applied led to reductions in maize height, ear appearing height, stem diameter, leaves number, and leaf size. This could be explained as an effect exerted by the N fertilizer on the maize cultivated in in-dyke alluvial soil. Moreover, plant height, ear appearing height, stem diameter, leaves number and leaf size in the treatments supplemented with MFPNSB had better results than those in treatments without supplemented treatments (Table 4), because N plays a key role in synthesizing protein, nucleic acid, and leaves chlorophyll (Shrivastav et al., 2020). Therefore, the sufficient supplementation of N accelerated the formation of chlorophyll, resulting in better photosynthesis and growth of hybrid maize (Table 4).

The result in Table 5 revealed that ear size values among the treatments fertilized with N fertilizer rate from 55 to 100% N of RFF were insignificantly different (Table 5). This finding was consistent with the study by Ngosong et al. (2019), where ear length and diameter differed unremarkably while applying high N fertilizer rates from 50 to  $200 \text{ kg N ha}^{-1}$ . However, with fertilizing 55% N of RFF plus MFPNSB, ear length reached 18.1 cm, higher than the length in the treatment fertilized with 100% N of RFF (17.4 cm) (Table 5). This result was also in accordance with previous studies, where both N-fixing bacteria and inorganic fertilizer interacted with each other and all contributed to improving yield components and N fertilizer use efficiency (Bargaz et al., 2018). No significant differences in were observed, seeds number per row among the treatments fertilized with N fertilizer rates from 55 to 85% N of RFF. However, a significant difference ( $p 0.0042 \times 10^{-19} < 0.05$ ) was found between the treatment fertilized with 55% N of RFF and the treatment with 100% N of RFF, and between the treatments supplemented with and without MFPNSB (Table 5). Furthermore, the application of N-fixing bacterial strains in combined with N fertilizer rates also raised the number of seeds per row, in comparison with no bacterial supplementation (Brum et al., 2016). Fertilizing 85% N of RFF plus MFPNSB gave ear length and seeds number per row of 18.2 cm and 43.8 seeds  $\text{row}^{-1}$ , respectively, which were different significantly ( $p 0.0025 \times 10^{-15}$ ,

$0.0042 \times 10^{-19} < 0.05$ , respectively) from the treatment fertilized with 100% N of RFF (17.4 cm and 40.3 seeds row<sup>-1</sup>, respectively) (Table 5; Fig. S1A, S1B, and S1C). Similarly, apart from maize, another crop has been also reported for the consistent result, where sesame has been improved by a *Rhodopseudomonas palustris* species, and the fertilizer requirement reduced by 25% (Khuong et al., 2023b)

In the case of no inorganic fertilizer applied, the treatment supplemented with MFPNSB, *R. palustris* VNW64, VNS89, TLS06, and VNS02 had grain yield of 67.2 g pot<sup>-1</sup>, while 40.8 g pot<sup>-1</sup> was the grain yield in the treatment without supplemented bacteria. This revealed that the N fixing bacteria from the air and provided it to maize (Table 2; Fig. 1), resulting in higher grain yield in the treatments supplemented with MFPNSB (Fig. 2). Furthermore, in-dyke alluvial soil was unable to provide a sufficient amount of N nutrient to maize (Table 1), due to the significant differences between the treatment without fertilized N and the treatment fertilized with 100% N of RFF (Fig. 2). The treatment fertilized with 0% N of RFF possessed a grain yield of 40.8 g pot<sup>-1</sup>, while its counterpart, the treatment fertilized with 100% N of RFF, had a yield of 83.0 g pot<sup>-1</sup> (Fig. 2). In the treatment fertilized with 55% N of RFF plus MFPNSB, the yield was equivalent to that in the treatment fertilized with 100% N of RFF, with 87.3 compared to 83.0 g pot<sup>-1</sup>, respectively (Fig. 2). This result agreed with the study by Ngosong et al. (2019), where grain yield was claimed to rise when inorganic N fertilizer rates was raised. Besides, in the study by Sakarika et al. (2020), PNSB strains improved soil fertility, so plants grew well and gained higher grain yield (Table 4; Fig. 2; Fig. S1D). Furthermore, the *Rhodopseudomonas palustris* PSB06 has been reported to be able to not only improve pepper yield, but also change the soil's microbial communities as well (Luo et al., 2023). In the near future, the bacteria in the current study can be applied via spraying on leaves. A study by de Oliveira Siqueira Lino et al. (2023) has used a strain of *R. palustris* for foliar application on mango and resulted positively.

## 5. Conclusions

Inoculating of MFPNSB strains, *Rhodopseudomonas palustris* VNW64, VNS89, TLS06, and VNS02 on maize seeds improved soil pH<sub>Water</sub>, N concentration in soil, N uptake in plant, plant height and grain yield of hybrid maize cultivated on in-dyke alluvial soil. The supplementation of PNSB strains of *Rhodopseudomonas palustris* VNW64, VNS89, TLS06, and VNS02 reduced an amount of 45% N of RFF but still maintained grain yield as that in the treatment fertilized with 100% N of RFF. This bacterial mixture should be further applied and investigated for its performance under field conditions. This should deliver a promising way to reduce the use of chemical fertilizers.

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